TWINNING AND REPRODUCTION OF TWINS IN PELMATOHYDRA OLIGACTIS

C. L. TURNER

Department of Biological Sciences, Northwestern University, Evanston, Illinois

This paper is written to extend the observations of the writer (Turner, 1950; 1952) on the reproductive potential in clones of *Pelmatohydra oligactis* and the regulation of spontaneous structural anomalies in the same species. A review of relevant literature and certain conclusions drawn from the literature are contained in the two articles and they will not be repeated here except for a few citations bearing directly upon the subjects of longitudinal fission and regulation.

Hyman (1928) states (p. 73) "that fission in hydra is not a normal method of asexual reproduction but a mode of regulation of previously existing abnormalities." The writer (Turner, 1952) observed (p. 107) that "the bifurcated condition of the apex which initiates longitudinal fission originates most commonly, and possibly exclusively, in buds which are in the latter stages of development and are still attached to the parent." Chang, Hsieh and Liu (1952) found that fission occurred in buds in a ratio of about one in a thousand. The writer has found since 1952 in large mass cultures and also in isolated strains that twinning in buds is fairly common and that true twinning originates only in buds. Conditions resembling twinning have been discovered in mass cultures but when these specimens are followed through day by day some of them have proved to be cases of actual twinning but others have proved to be specimens undergoing regulation. The complexes are often resolved by fusion of the members, absorption of one member by the other or one member forms a new foot and separate from the other.

The term "twinning" is used here advisedly and has most of the features common to monozygotic twinning in higher forms. In both hydra and higher forms an embryonic mass, produced asexually as a bud in hydra, divides and two individuals proceed separately to develop into whole equal animals. The separation of the two individuals may be incomplete in higher forms and produce monstrosities in various degrees. In hydra, individuals will become separated eventually because they have retained to a remarkable degree the capacity for regulation during which multiple complexes are reduced to simple individuals with the characteristics of the parent animal. In hydra the initiation of twinning may occur at any stage of bud development but it is not completed by the time of detachment from the parent. Another process comparable to twinning in its end result is double budding. In this process two buds in precisely the same stage of development arise at the same time in the budding zone of the parent as separate masses, usually on opposite sides of the body stalk, and after developing at the same rate they become detached at the same time. At no stage are they connected and they differ from the ordinary bud in that ordinary buds arise in a sequence, differ from each other in stages of development and become detached from the parent at different times.

Materials and Methods

Specimens were separated from a large mass culture and were placed individually in bottles of about 100 cc. capacity. They were fed with Entomostraca daily and the water in which each lived was replaced daily with water from the large tank in which the mass culture was maintained. Daily records were kept of each specimen concerning its behavior, its reproduction and its periods of physiological depression.

In order to secure an accurate determination of the extent of twinning in the members of the mass culture, single non-twinning specimens were separated from the mass culture and a record was kept of all of the young produced by budding. The record of each was followed for 20 to 30 days and the proportion of twinning to non-twinning buds was determined from the totals. The process was repeated three times at two-month intervals.

When twinning buds were discovered they were isolated after they had become detached from the parents and daily records were kept of buds produced by the single undivided portions and by the bifurcated portions of the twin. The total number of buds produced by the complex was secured in this way and a comparison could be made between twinning individuals and single individuals of the rate of bud production. The proportion of single to twinning buds was obtained and could be compared to the production of single and twinning buds in control specimens which had no previous history of twinning. The possibility that certain strains had a tendency to produce a high proportion of twinning buds could be examined from the results.

Proportion of Twinning to Non-twinning Individuals

Any estimate of the proportion of twinning to non-twinning individuals based upon bifurcated specimens taken from a large mass culture would be invalid because a part of the individuals in the bifurcated state would not be cases of genuine twinning. Some of them would be mature individuals in which a bifurcation had arisen at the apex during or immediately after a period of depression. By the selection of healthy single individuals and the recording of each bud which arose in these individuals, it was possible to know whether any suspected case of twinning was real and, if it was real, to know also the place of its origin and its subsequent history. Twenty-five single individuals selected from the large mass culture were given the most favorable conditions for reproduction and within a period of 20 to 30 days they produced a total of 705 buds of which 14 were twins. The twinning buds represented 1.98 +% of the total. Two months later 11 single individuals, selected from the same mass culture and maintained in the same way, produced 274 buds of which 4 (or 1.45 +%) were twins. The operation was repeated two months later and 12 specimens produced 299 buds of which 6 (or 2.01%) were twins. In the three samples, 48 isolated individuals produced 1278 buds of which 24 or 1.87 +% were twin buds.

Twenty of the twin buds were isolated when they were detached from the parent and a record was kept of the production of buds by each specimen for 13 to 22 days. A total of 523 buds was produced of which 19 (3.63 +%) were twin buds. During this time ten of the twinning specimens produced only single buds but one of them produced three twinning buds (Fig. 3) and several produced two twinning buds

(Figs. 1 and 2). Since the reproducing individuals derived from twinning buds produced nearly twice as many twinning buds as individuals taken at random from the mass culture, it may be inferred that an inherited tendency for bud twinning existed in the strains showing unusual bud twinning.

Origin and Structure of Twins

Twinning has been observed in buds at various stages of development. In the earliest cases the bud develops a bilobed condition almost from the moment of its origin as a bud (Fig. 17). Each lobe then elongates and differentiates (Figs. 4 and 5) and by the time of detachment from the parent the two members of the twin are separate except at the base (Fig. 6). If the specimen indicated in Figure 6 were to be found in a mass culture without its previous history being known, it might be interpreted as a case of apico-basal fission but it is actually one in which no fission is involved up to the time of its detachment. Rather, two separate portions of the original single bud have undergone parallel development. After detachment the twinning individual undergoes fission and the two members are separated. It may be stated that twinning gives rise to an anomalous state which is resolved by regulation (fission).

A bud may develop as a single unit for some time and then give rise to dual masses at the apex (Fig. 7). The basal single portion elongates somewhat and at the same time the members of the divided portion will elongate and differentiate. After detachment from the parent (Fig. 8) fission of the remaining common stalk and the base occurs. Regulation (fission) is involved to a greater extent than it is in cases of very early bud-twinning.

Twinning occurs at a late stage of bud development in some cases (Fig. 9) and when the bud is detached from the parent only the apical portion of the bud is bifurcated. The regulation process (fission) occupies a longer part of the entire period during which two complete individuals are formed from a single bud.

A bud occasionally gives rise to three instead of two units. In the case illustrated in Figure 11 three hypostomes, each with a circle of tentacles, arose in a late stage of bud development. Before the bud was detached the hypostome of one unit was absorbed by another but the tentacles of the absorbed unit remained intact. When the bud was detached it resembled the one shown in Figure 10 except that one of the terminal units possessed supernumerary tentacles. In this case three processes of regulation would be involved before two normal single individuals were formed from the complex bud. Absorption of the hypostome occurs before bud detachment. Apico-basal fission would separate the two members after detachment of the complex from the parent, and still later fusion of tentacles would occur in the individual having supernumerary tentacles until the normal number of tentacles was produced.

Secondary twinning, *i.e.*, twinning in one or both members of a specimen which is itself a twin, has been observed a number of times. In Figure 12 a single specimen is illustrated together with a twinning bud. The primary twinning occurred when the bud was half developed and the two members of the twin bud proceeded to elongate and differentiate. At a late stage in differentiation one of the members divided at the apex. When the bud was detached it consisted of a common stalk and foot and a stalk divided in its terminal half. Also one member of the di-

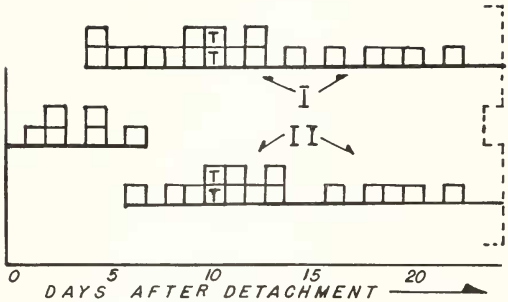


FIGURE 1

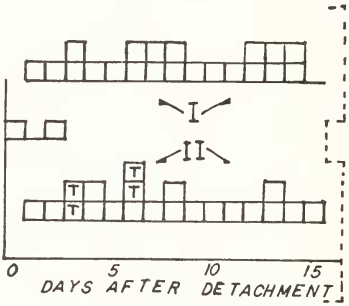


FIGURE 2

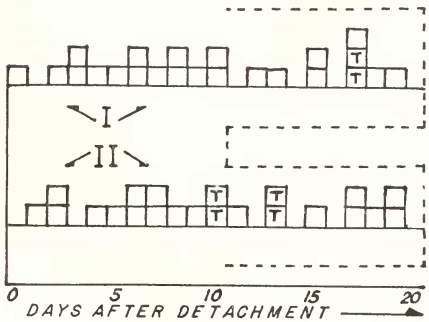


FIGURE 3

FIGURE 1. Reproduction by budding in twinning specimen shown in Figure 10. Period covers time from detachment from parent to time of separation of twins by apico-basal fission. Fission was completed in 24 days. Squares indicate new buds per day. Reproductive record of single portion shown at left, of divided portions, in I and II. T indicates twinning in a bud.

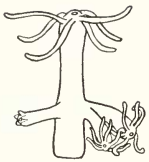
vided portion had two hypostomes. In the member which was divided at the end, fusion occurred and within several days a single hypostome was present, surrounded by eight tentacles. Fission separated the two primary members eighteen days after detachment of the twinned bud from the parent. Figures 15, 16 and 17 illustrate cases in which twinning buds had become detached from the parent and were actively reproducing. Fission which will separate the primary twins is in progress. A new bud appearing on one of the stalks of the specimen (Fig. 17) is twinning in an early stage producing a case of secondary twinning. Similar situations are illustrated in Figures 15 and 16 except that in both cases fission has divided the detached primary twin down through the budding zone and the secondary twinning buds are arising from divided instead of the common undivided parts of the stalk. A remarkable case is illustrated in Figure 18. The complex of four members arose as a single bud which twinned at an early stage. After a short period of elongation of the two members each member twinned again. Elongation of each of the four members occurred and each differentiated with a full complement of tentacles and a hypostome. After the complex was detached from the parent the primary division was completed by fission and some days later a secondary fission separated the members of the secondary twins. No fusion of any kind occurred. It appears in comparing the cases shown in Figures 9, 10 and 18 that secondary twinning is likely to be effective and to produce separate individuals if it occurs early and the members are able to grow and to differentiate a full complement of normal parts, but if the twinning occurs very late one member is likely to be absorbed by the other.

Complexes are encountered occasionally which involve at the same time multiple twinning and other unusual features. They present a complicated appearance and the units can be accounted for only if a complete record has been kept from the time of origin of the individual producing the complex. A case in point is the complex illustrated in Figure 16, and the history of this case is as follows. A bud arose from a normal single hydra and proceeded to twin when half developed. The twinning bud became detached from the parent and began to produce buds of its own in the common stalk almost immediately. Fission of the twinned individual was carried basally and passed through the budding zone. At the moment illustrated in Figure 16, I and II represent the divided portions of the individual described. Portion I has three buds, A, B and C. Bud C is going through the unusual process of giving rise to a new bud before it has become detached from the parent. Portion II is giving rise to buds A′, B′ and C′. Bud C′ twinned at an early stage and has not become detached. The subsequent history of the complex involved further reproduction and a resolution into single normal units. Portions I and II gave rise to new buds while they were being separated by fission and thereafter appeared as normal single budding individuals. Bud C became detached within a few hours and became a single reproducing individual. Twinning bud C′

The notch in the broken line at the right indicates the time of separation by fission of the members of the twin.

FIGURE 2. Reproduction by budding in twinning specimen divided for half of its length when detached from parent as in Figure 8. Fission complete on 16th day. Symbols as in Figure 1.

FIGURE 3. Reproductive record in twinning specimen divided for ⅞ of its length at time of detachment from parent. Reproductive period extends from time of detachment of twinned bud from parent (0 days) to completion of fission (10 days) and in addition, 10 days after completion of fission. Symbols as in Figure 1.

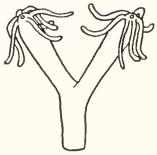


4

5

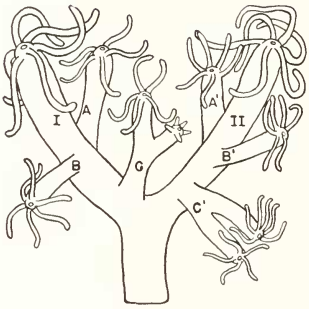
6

7

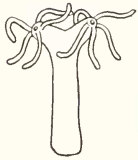


8

9

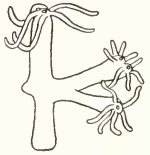


16

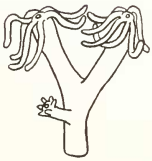


10

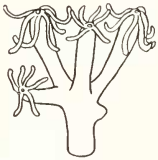
11



12

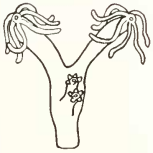


13

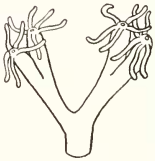


14

15



17



18

FIGURES 4–18.

became detached in several hours and nine days later fission had separated the members of the twin. In the meantime twinning bud C′ was budding off new individuals. A total of eighteen days were required from the time of the appearance of the original bud for the formation of the complex and for its resolution into single individuals.

Reproduction in Twins

Reproduction in twins was studied for the purpose of determining whether the process of twinning and subsequent regulation interfered with, or in any way affected, the process of reproduction and also to compare the over-all production of new individuals in twins with that of single individuals. Three cases were selected for illustration on the basis of the degree of apico-basal division in the twins at the time of the detachment from the parent.

In case 1 (Fig. 1) the twinning bud was detached from the parent when the apical part was divided for approximately one-third of the entire length of the bud. The budding zone in the common stalk was not divided at the time of detachment and three individual buds were produced within the first four days on the common stalk. Buds produced by the common stalk are shown by blocks in the middle line of the three lines of blocks. On the fourth day after detachment, fission

Figure 4. Single parent with bud which twinned at an early stage and developed as two individuals separated except for basal ⅛ at time of detachment.

Figure 5. Single parent with bud which twinned at an early stage and was almost completely divided at time of detachment from parent.

Figure 6. Bud shown in Figure 4 after detachment from parent.

Figure 7. Young bud which twinned when half developed.

Figure 8. Bud shown in Figure 7 after detachment from parent.

Figure 9. Bud which twinned at a late stage of development. Supernumerary tentacles and fusing pairs of tentacles in parent indicate that parent is an incompletely regulated specimen in which two apical units have fused. Bud was divided for ⅛ of its length when detached.

Figure 10. Bud shown in Figure 9 after detachment from parent. Apico-basal fission was completed in 24 days. See Figure 1.

Figure 11. Multiple division of the apical end of a bud. After detachment one of the hypostomes fused with the nearest hypostome. The two remaining units were separated by apico-basal fission.

Figure 12. Twinning occurred at an intermediate stage of bud development and secondary twinning occurred late in the development of one of the units. The secondary twinning was reduced by absorption of one member by the other.

Figure 13. Budding in the undivided portion of a specimen which twinned as a bud and is undergoing apico-basal fission. See Figure 2.

Figure 14. Same specimen as in Figure 13. Apico-basal fission has proceeded down to the budding zone.

Figure 15. Same specimen as in Figure 13. Apico-basal fission has proceeded through the budding zone down to the stalk. One of the new buds is a twin.

Figure 16. Specimen 8 days after it was detached from parent as a twinned bud divided for about ½ of its length. Apico-basal fission has proceeded downward through the budding zone. Member I has three buds, A, B and C. C, not yet detached, is producing a bud. Member II has produced three buds, A′, B′ and C′. C′ is a twinning bud. Specimen produced 43 buds before fission was completed in 21 days.

Figure 17. Specimen which arose as a twinned bud and is now producing a twinning bud. Divided members of the new bud have unusual arrangement along apico-basal axis of parent.

Figure 18. Complete double twin. Specimen twinned in an early bud stage and each member twinned at a later stage before the four-member complex was detached from the parent.

had carried down to the budding zone and two new buds appeared on the common stalk and two upon one of the divided portions above the point of function with the other member. On the fifth day after detachment another bud appeared on the same member. On the sixth day one new bud appeared upon the common stalk and one each on the divided members. Thereafter no new buds appeared upon the common stalk and it is apparent that fission had carried down through the budding zone and that the time for its passage through the budding zone was three to four days. The time of the complete separation of the members of the twin was 23 days after the twinned bud had become detached from the parent. The time of separation is indicated by the notch in the broken line at the right of the figure. During the process of fission, during which the members were separated from each other, reproduction continued in each member at approximately the same rate as it would have occurred in a single individual.

Reproduction in a bud which had twinned somewhat earlier than the one shown in Figure 1 is represented in Figure 2. When the bud was detached from the parent it was divided apically for about one-half of its length, presenting the appearance of the specimen shown in Figure 8. The division point was within the budding zone and each member began to form new buds the day after the twin was detached from the parent. Fission carried down through the budding zone within three days during which time the common stalk produced two buds. The members of the twin were separated from each other 16 days after the twinned bud was detached from the parent and each produced buds continually during the regulatory process of fission.

A twin-bud specimen which was divided for about seven-eighths of its length when detached from the parent reproduced as shown in Figure 3. The specimen (Fig. 6) had twinned at an early stage and separate and complete budding zones were represented in each member. Each member began to give off new buds as soon as the specimen was detached from the parent and continued to do so for the ten days required for fission to separate the members. The reproductive history of each member for ten days after separation is shown at the right in the diagram. It will be noted that the rate of bud production in each member was about equal and that the rate is the same whether the members were attached to each other or separated.

It is apparent from the results that the process of twinning, and of fission which separates the members of a twinned individual after its detachment from the parent, do not affect the process of reproduction by budding. It may be added that there is no interference with the formation and maturing of spermaries. Buds, whether single or twinning, become detached from the parent within 48 hours after their first appearance if the parent is not in a state of depression, and spermaries are not formed until later. However, spermaries have been observed in both members of twinned specimens during the process of fission which later separates the members of the twin.

Duration of Fission

Fission, as the term is used here, refers to the process moving in the apico-basal axis by which the members of a twinned individual are separated. It occurs after the bud has been detached from the parent. The rate at which fission proceeds is

quite variable and the duration of the process depends upon the degree of initial separation of the members at the time of detachment from the parent and upon the rate at which it proceeds. Four individuals divided at the apical end for a distance of one-fifth of the total length required, respectively, 17 days, 27 days, 38 days and 51 days for complete separation. The process moved through the apical third rapidly, and proceeded through the budding zone in three to four days. Fission moved at a slower rate through the body stalk at the basal end and lagged greatly in the region of the foot. In an extreme case 20 days were required for separation of the foot after fission had carried down to that point. Two specimens, each separated for one-half of the total length at detachment, required 23 days for the completion of fission. Three specimens divided almost to the base at the time of detachment required, respectively, 8, 9 and 11 days for complete separation.

Summary

1. In the specimens observed in pedigreed cultures, genuine twins arose only in buds.

2. Fission, regarded as a separate regulatory process in which twinning complexes are resolved into single individuals, occurs after the twinning buds have become detached from the parent.

3. In pedigreed cultures specimens arising as single buds produced 1278 buds of which 24 were twin buds. Specimens arising as twinning buds produced 523 buds of which 19 were twin buds.

4. Twinning may occur in a bud at any stage of development. An early twinning bud is deeply divided at the time of detachment from the parent and a late twinning bud is divided only at the apex.

5. Multiple twinning occurs occasionally in which one or both members of a twin bud undergo secondary twinning before detachment of the complex from the parent.

6. Bud production by a specimen arising as a twin bud is equal to that of a single individual as long as the budding zone is undivided. Bud production is doubled as the budding zone is divided by fission.

7. Completion of fission of a twin bud requires usually from 8 to 27 days but may take as long as 51 days in a depressed specimen. Fission proceeds rapidly at the apical end, passes through the budding zone in three or four days and is retarded most at the basal end of the body stalk and the foot.

LITERATURE CITED

Chang, Joseph T., H. H. Hsieh and D. P. Liu, 1952. Observations on hydra, with special reference to abnormal forms and bud formation. *Physiol. Zool.*, **25**: 1–10.

Hyman, Libbie H., 1928. Miscellaneous observations in Hydra, with special reference to reproduction. *Biol. Bull.*, **54**: 65–109.

Turner, C. L., 1950. The reproductive potential of a single clone of *Pelmatohydra oligactis*. *Biol. Bull.*, **99**: 285–299.

Turner, C. L., 1952. The regulation of spontaneous structural anomalies in *Pelmatohydra oligactis*. *Biol. Bull.*, **103**: 104–119.